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PERSPECTIVES IN BASIC SCIENCE

# Ear and kidney syndromes: Molecular versus clinical approach

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**Ear and kidney syndromes: Molecular versus clinical approach.** The association between ear and kidney anomalies is not usually due to an insult to the embryo. In recent years, many essential development control genes that coordinate the assembly and function of kidney and ear have been discovered through the generation of animal mutants and have increased our understanding of the mechanisms of human oto-renal diseases. Here, we describe ear and kidney clinical syndromes and their molecular expression.

First ear and kidney abnormalities were reported in 1946 by Edith Potter's association of crumpled and flattened ears with bilateral kidney agenesis [1]. Ear malformations are associated with an increased frequency of clinically significant structural renal anomalies compared with the general population. These include specific multiple congenital anomaly syndromes, Townes-Brocks syndrome (TBS), branchio-oto-renal (BOR) syndrome, among others. Many studies in the literature have noted a significant association between renal anomalies and various ear anomalies. Ear pits and tags, perhaps the most common minor ear malformations, occur with a frequency of five to six per 1000 live births [2, 3]. In the pediatric population, structural renal anomalies occur in one to three per 100 live births [4]. Children who had isolated preauricular tags presented on renal ultrasonography urinary tract abnormalities in 3% to 8% of cases as hydronephrosis, horseshoe kidney, kidney agenesis, or hypoplastia [5, 6]. A recent study [7] of 32,589 consecutive live births, still births, and abortions over 10 years in the Mainz Congenital Birth Defect Monitoring System noted a 1.2% prevalence of renal anomalies. After patients with syndromic diagnoses were excluded, auricular pits or cup ears and specific renal anomalies were still associated [7].

Half a century later, ear and kidney development has been characterized in great detail, demonstrating that embryologically, ear and kidney primordia arise at different times and develop at different rates (Fig. 1). Therefore, the association between ear and kidney anomalies is usually not due to an isolated insult to the embryo that would affect both developing structures at the same time. The genes responsible for hereditary deafness in humans and in vertebrate model systems have been recently identified. These serve as useful molecular markers, but more important, a number of these genes undoubtly play key roles in kidney and ear determination. Table 1 summarizes the findings in the various model systems to date. Recent identification of genes that are responsible for BOR syndrom and TBS, along with gene expression studies of these genes, has shown that these genes were expressed in developing ear and kidney structures at different times during morphogenesis [8].

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## EAR ANATOMY

The ear is the organ of hearing and balance and consists of three parts: the outer ear, the middle ear, and the inner ear. The outer ear and middle ear are the apparatus for the collection and transmission of sound. The inner ear is responsible for analyzing sound waves, and also contains the mechanism by which the body keeps its balance. The outer ear is comprised of the pinna and ear canal; the middle ear includes the eardrum, hammer, anvil, stirrup, and eustachian tube; and the inner ear includes the vestibule, semicircular canals, and cochlea. Sensory impulses from the inner ear pass to the brain via the vestibulocochlear nerve.

### Outer ear

The outer ear consisting of the pinna (also called the auricle) is the visible part of the ear and is composed of folds of skin and cartilage. The pinna leads into the ear canal (also called the meatus) and is about 1 inch (or 2.5 cm) long in adults and closed at its inner end by the tympanic membrane or (eardrum). The part of the canal nearest the outside is made of cartilage. The cartilage is covered with skin that produces wax, and the tiny hairs in the canal traps dust, pollen, pollution, and small foreign bodies.

### Middle ear

The middle ear is a small cavity between the eardrum and the inner ear. It conducts sound to the inner ear by means of a chain of three tiny, linked, movable bones called ossicles. They link the eardrum to an oval window

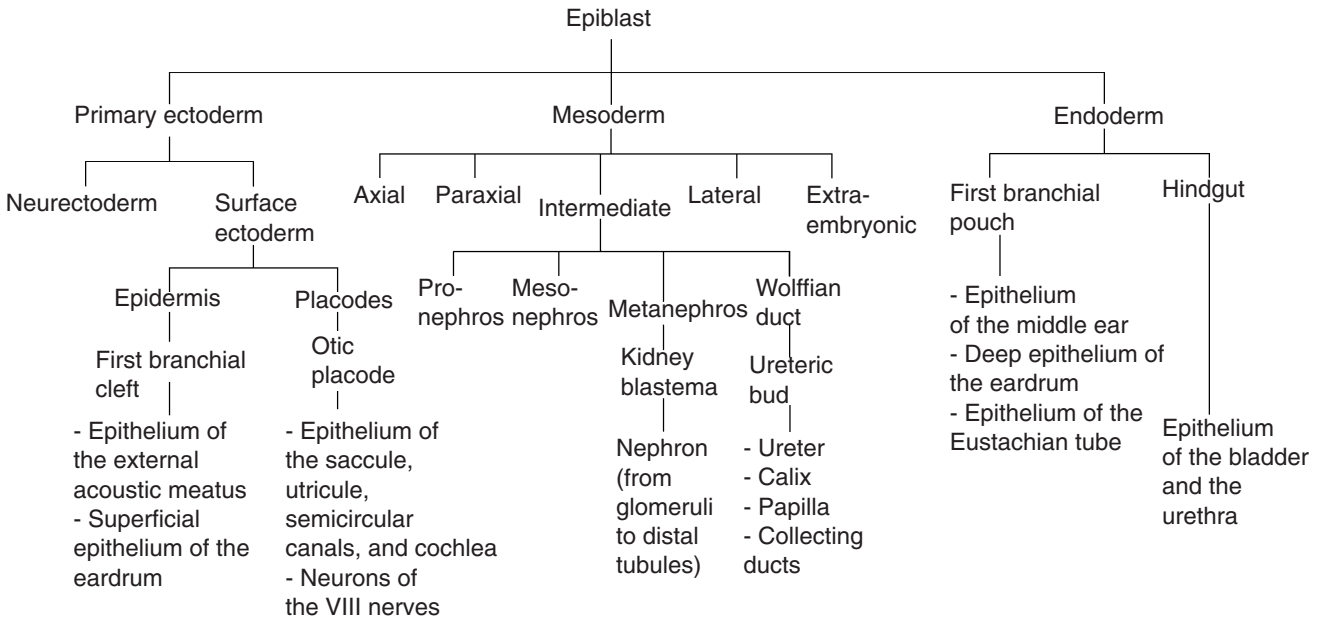


**Fig. 1. Ear and kidney embryology.**

in the bony wall on the opposite inner side of the middle ear cavity. The bones are named because of their shapes. The malleus, or hammer, is joined to the inside of the eardrum. The incus, or anvil, has one broad joint with the malleus (which lies almost parallel to it) and a delicate joint to the third bone, the stapes, or stirrup. The base of the stapes fills the oval window, which leads to the inner ear. The middle ear is cut off from the outside by the eardrum, but it is not completely airtight. A ventilation passage, called the eustachian tube, runs forward and down into the back of the nose. The eustachian tube is normally closed, but it opens by muscular contraction when yawning and swallowing. The middle ear acts as a transformer. It passes the vibrations of sound from compression and decompression of the outside air. The air is a thin medium that carries the sound into the inner ear where the fluid in the inner ear, a thicker medium, resonates the sound vibration.

### Inner ear

The inner ear is an extremely intricate series of structures contained deep within the bones of the skull. It consists of a maze of winding passageways, collectively known as the labyrinth. The front part, the cochlea, is a tube resembling a snail's shell and is related to hearing. The rear part, which is three semicircular canals and two other organs, is concerned with balance. The semicircular canals are set at right angles to each other and are connected to a cavity known as the vestibule. These canals contain hair cells bathed in fluid. Some of these cells are sensitive to gravity and acceleration and others respond to head positions and movement (side to side, up and down, or tilted). Posture or direction information is registered by the relevant cells and conveyed by nerve fibers to the brain.

The ear is susceptible to a large number of disorders, some of which can lead to deafness.

## FOCUS ON EAR AND KIDNEY EMBRYOLOGY: MOLECULAR MECHANISMS AND GENE EXPRESSION

The embryogenesis of the association of renal and auricular anomalies is unclear. The combination of ear and kidney anomalies in the early stages of development can be explained on the assumption that mesodermal induction (transcription factor, gene expression) is responsible for normal differentiation of both organs. However, in the late stages of development, those factors only intervene in the inner ear function (Fig. 2).

Organogenesis involves many cellular processes, such as proliferation, cell adhesion, apoptosis, cell differentiation, cell migration, all of which require molecules from different classes and family. In recent years, many essential developmental control genes that coordinate the assembly and function of kidney and ear have been discovered through the generation of animal mutants and have increased our understanding of the mechanisms of epithelial interactions.

Transcription factors, growth factors, and their receptors that are essential for both ear and kidney development are discussed and their functions and phenotype are summarized in Tables 1 and 2, respectively:

### Pax-Six-Eya-Dach signaling network

A number of transcription factors are expressed in the presumptive otic ectoderm, but two of the earliest

**Table 1.** Ear and kidney genes' functions

| Genes | Ear and kidney genes' functions | |
|---|---|---|
| | Ear | Kidney |
| Glial cell-line–derived neutrophic factor | Protects hair cells from damage | Essential for ureteric bud growth and branching morphogenesis of the ureteric bud epithelium |
| Fibroblast growth factors | Induces both the otic placode and the epithelial organization of the otic vesicle | Maintains the nephrogenic mesenchyme; induces its condensation and autocrine secretion of Wnt-4 converts it to epithelium |
| Bone morphogenic proteins | Proteins emanating from the otic epithelium influence chondrogenesis of the otic capsule, including the cartilage surrounding the semicircular canals | Modulate ureteric bud branching and keep bud development in step with that of other tissue types |
| Wnt signaling and Frizzled receptors | Could be involved in several aspects of late cochlear differentiation and/or auditory function | Critically required for tubulogenesis in the pronephric kidney |
| GATA3 | Repressor of critical genes involved in cell differentiation in the organ of corti | Intervenes at the interface of Wolffian duct and the metanephric blastema at about 7 weeks gestational age |
| Parchorin | Generally plays a critical role in water-secreting cells, possibly through the regulation of chloride ion | |
| Prestin | Required for electromotility of the outer hair cell and for the cochlear amplifier; the motor protein of the cochlear outer hair cell | |
| Atrial natriuretic peptide | May be involved in fluid homeostasis in the inner ear and the kidney | |
| Barttin | Crucial for renal salt reabsorption and potassium recycling in the inner ear | Increases surface expression and changes current properties of ClC-K channels and is required for adequate tubular salt reabsorption |
| ATP6B1 | Role in endolymph pH homeostasis and in normal auditory function | Role in normal vectorial acid transport into the urine by the kidney encoding the B-subunit of the apical proton pump mediating distal nephron acid secretion |
| Gyro | Intervenes on renal excretion of phosphate, and impairment of Na+/phosphate cotransport by renal brush-border membrane vesicles | |
| KCNQ | May be present in amniote vestibular hair cells; destabilization of the resting potential and increase in [Ca2+]i, as may result from impaired KCNQ4 function in IHCs, whereas mutations in KCNQ1 causes deafness by affecting endolymph secretion, the mechanism leading to KCNQ4-related hearing loss is intrinsic to outer hair cells | |
| AQP-2 | Role in the development of endolymph homeostasis | Regulated urinary diluting ability; important for rapid near-isosmolar transepithelial fluid absorption/secretion and for rapid vectorial water movement driven by osmotic gradients |
| Mpv17 | Peroxisomal protein involved in the metabolism of reactive oxygen species; the severe sensorineural hearing loss and degenerative changes of the cochlear structures indicate that cochlear structures, especially the outer hair cells and the intermediate cells of the stria vascularis, are vulnerable to the missing Mpv17 gene product; both organs have specialized epithelia involved in active ion transport, which are separated from the vessels by a basement membrane of similar composition; glomerular and the stria vascularis basement membrane are simultaneously affected in early stages | |

genes activated in the presumptive otic ectoderm are the transcriptional regulators *Pax8* (OMIM 167415) and *Pax2* (OMIM 167409) [9–11]. Other transcriptional regulators expressed in preplacode otic ectoderm include members of the *Six, Eya,* and *Dach* gene families, defined as a Pax-Six-Eya-Dach synergistic regulatory network involved in the formation of a number of organs. Metanephric kidney development begins with the formation of the metanephrogenic mesenchyme; this event depends on the prior action in the intermediate mesoderm of transcription factors such as *Pax2* and *Eya1*.

*Six1* (OMIM 601205) and *Six4* (OMIM 606342) are expressed in the developing inner ear. Expression of *Six* genes is also seen in the individual otic placodes in the Xenopus, chick, and mouse [12–16].

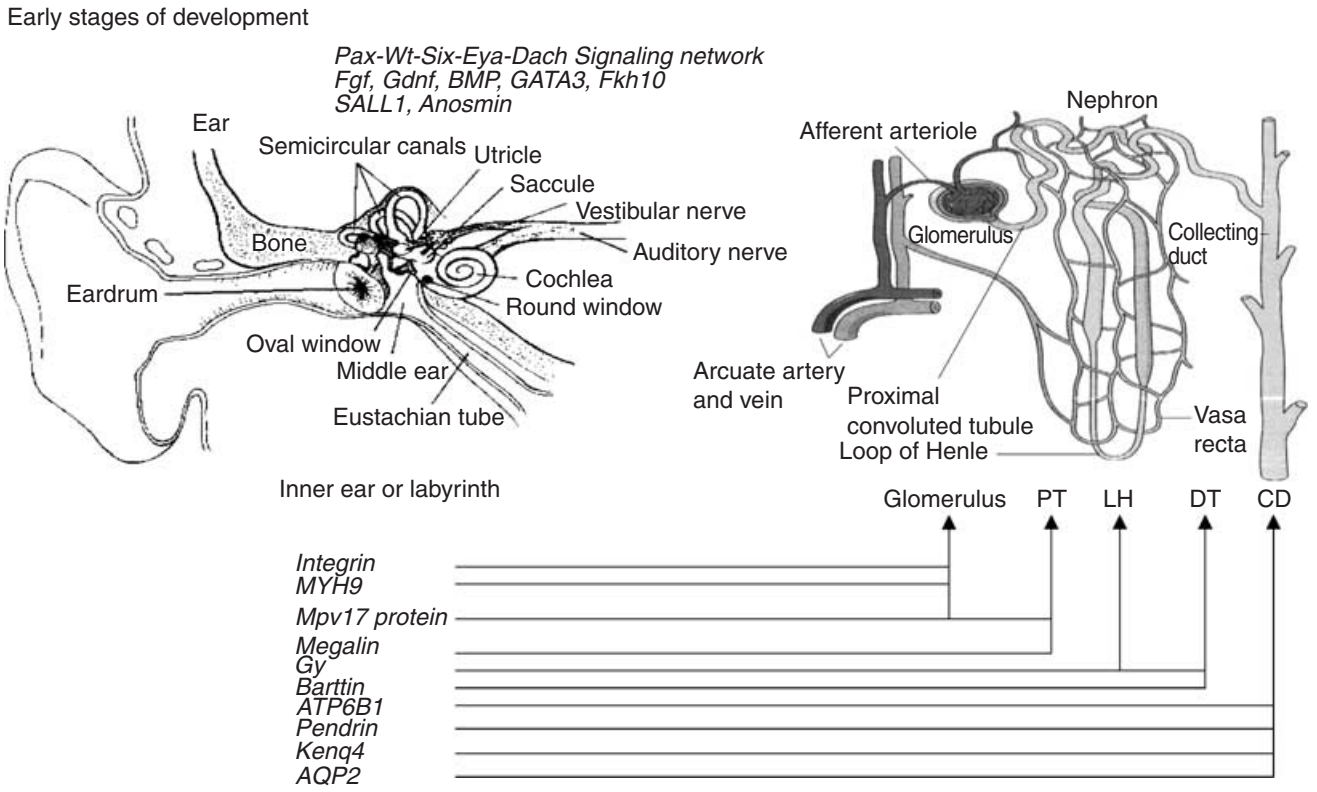


**Fig. 2. Focus on anatomy, physiology, and cell biology.**

In the embryonic human kidney, the *EYA1* gene is expressed strongly, and in the BOR syndrome there is an inductive fault between the ureteric bud and the metanephric mesenchymal mass as the ureteric bud branches into the renal parenchyma. *Eya4* is also thought to have a role in ear formation, as its expression has been reported in neuroepithelia of rat inner ear from E14.5 onwards, and loss of *Eya4* in humans causes deafness [17, 18].

Dach proteins (OMIM 603803) have shown to have direct protein-protein interactions with Eya family members [19] and epistatic analyses in *Drosophila* have positioned dachshund (dach)—the *Drosophila* homologue of the vertebrate *Dach* genes—downstream of eya. During ear development, *Dach1* overlaps with *Eya1* and *Pax2* expression but *Dach1* expression does not depend on these genes, as its early expression is not affected in the null mutants.

### Glial cell-line–derived neutrophic factor

Glial cell-line–derived neutrophic factor (GDNF) encodes a member of the transforming growth factor-β (TGF-β) family of signaling molecules, which has an important role in ureteric-bud induction. Its loss in gdnf-knockout mice leads to the failure of ureteric-bud formation [20].

### SALL1

Sall1 is a mammalian homologue of the *Drosophilia* region-specific homeotic gene splat. Sall1 is expressed in the kidney mesenchyme and its inactivation leads to incomplete ureteric-bud growth and to the failure of tubule formation [21]. Heterozygous *SALL1* mutations in humans lead to TBS.

### Fibroblast growth factors

Fibroblast growth factor (FGF) receptors (FGFRs) were localized to all nephron and collecting duct epithelia. *FGFR-1* and *FGFR-3* were localized to glomeruli, *FGFR-3* to proximal tubules and *FGFR-1* to thin limbs. *FGFR-1* through *FGFR-3* was localized to distal straight tubules, with *FGFR-1* and *FGFR-3* localized to distal convoluted tubules. *FGFR-1* and *FGFR-3* were localized to medullary collecting ducts. In addition, *FGFR-1* was localized to the smooth muscle of renal arteries. All FGFR variants were expressed in the cortex and outer medulla, with fewer FGFRs in the inner medulla. *FGF-1*, *FGF-2*, *FGF-7*, *FGF-8*, and *FGF-9* were expressed in the kidney, with FGF-10 expression found only in the cortex. [21]. Experiments by Represa et al [22] implicated FGFs as having an important early role in ear formation. Depletion of *Fgf3* in explants by antisense nucleotides and antibodies prevented the formation of ear vesicles, whereas

**Table 2.** Ear and kidney syndromes

| Genes, gene products, and chromosome | Developmental impact | | Lesions | | Clinical syndrome |
|---|---|---|---|---|---|
| | Ear | Kidney | Ear | Kidney | |
| Pax-Six-Eya-Datch | Preplacode otic ectoderm; neuroepithelia of inner ear | Metanephrogenic mesenchyme; urogenital ridge; ureteric bud outgrowth | Hearing loss; cervical fistulas and cysts; preauricular pits and appendages; auricular malformations; atresia to stenosis of the external auditory canal; underdeveloped cochlea and semicircular canals | Collecting system duplications; renal hypoplasia; cystic dysplasia and agenesis; hydronephrosis; ureteropelvic junction obstruction; vesicoureteral reflux; glomerular hyalinization; mesangial proliferation; basement membrane splitting | Branchio-oto-renal (BOR) syndrome |
| SALL1<br>Chr 16q12.1 | Later steps in development of the outer, middle and inner ear; differenciation of the otic vesicle | Outgrowth of the urorectal septum; metanephric mesenchyme | External ear anomalies; hearing loss | Dysplastic kidneys or agenesis; horseshoe kidney; multicystic kidney; posterior urethral valves; Vesicoureteral reflux; renal failure | Townes-Brocks syndrome (TBS) |
| Anosmin1<br>Chr Xp22.3 | Inner ear | Mesonephric tubules and duct; branches of ureteric bud | Hearing loss | Renal aplasia; absent development or early degeneration of the collecting duct system | Kalmann syndrome |
| Barttin<br>Chr 1p31 | Stria vascularis ($K^+$ secreting marginal cells); $K^+$ secreting vestibular dark cells | Stria vascularis; thick ascending limb; basolateral membranes of intercalated cells of the collecting duct | Sensorineural deafness | Renal failure; diabetes insipidus; renal salt wasting | Bartter sensorineural deafness (BSND) syndrome |
| Pendrin<br>Chr 7q31 | Inner ear | Apical membrane of intercalated cells in the cortical renal collecting duct | Developmental abnormalities of the cochlea; sensorineural hearing loss | Acid-base disturbances? | Pendrin syndrome |
| MYH9 gene | Inner ear | Glomeruli | Sensorineural hearing loss | Nephritis; proteinuria; hematuria | Fechtner syndrome |
| ATP6B1 gene | Cochlea and endolymphatic sac | Encodes the B subunit of the apical proton pump mediating distal nephron acid secretion | Sensorineural hearing loss | Severe hyperchloraemic metabolic acidosis in childhood; hypokalaemia; decreased urinary calcium solubility, | Autosomal-recessive distal renal tubular acidosis |
| Fibroblast growth factor<br>Chr11q | Endolymphatic appendage; otic placode; regulates pillar cell development | All nephron; all tubular duct; in cortex and outer medulla; smooth muscle of renal arteries | Severe ear malformations | Renal tubular dysfunction?; small kidney; less ureteric bud branches and nephrons | ? |
| Integrin | Apical hair cells surface stereocillia maturation | Ureteric bud induction; metanephric mesenchym; mesenchymal-epithelial transition | Sensorineural hearing loss, | Recurrent hematuria; ultrastructural changes of the glomerular basement membrane; renal failure | Alport disease |
| Forkhead (FKHL)<br>Chr 5q34 | Otic vesicle; cochlea; vestibulum | Later stages of kidney development | Inner ear malformations | No kidney dysfunction? | "Common cavity" phenotype |
| Megalin<br>Chr 2q24-q31 | Inner ear | Glomeruli; proximal renal tubule | Hearing loss | Tubular resorption deficiency with excretion of low molecular proteins; Fanconi syndrome? | Nephro and ototoxicity of antibiotics |
| Na/Pi cotransporters<br>PHEX<br>Chr Xp22.2-p22.1 | Inner ear | Brush-border membrane of renal proximal tubular cells | Inner ear abnormalities; deafness; hearing loss | Hypophosphatemia; kidney stone | X-linked hypophosphatemia |
| Bone morphogenic protein (BMP4)<br>Chr 14q22-q23 | Otic epithelium of inner ear; otic capsule; cartilage of semicircular canals | Metanephros ureteric branching morphogenesis; periureteric smooth muscle layer and ureteric elongation | ? | Hypo/dysplastic kidney; hypoureter; ectopic uretrovesical junction; double collecting duct | ? |
| Wnt4<br>Chr 1p35 | Inner ear | Distal collecting duct epithelium; tubulogenesis; mesenchyme-to-epithelium transformation | Otic malformations | Abnormal development of the kidney; failure of kidney-tubule formation; renal fibrosis | ? |
| GATA3<br>Chr 10p15 | Otic vesicle | Branching morphogenesis; ureteric bud; collecting duct system; mesangial cells | Sensorineural deafness | Renal malformations | Hypoparathyroïdism, sensorineural deafness, and renal anomalies (HDR) syndrome |

the application of *Fgf2* was shown to rescue otic vesicle induction in explants where the hindbrain had been removed. A later role for *Fgf3* signaling in inner ear formation was postulated given that homozygous *Fgf3* knockout mice had severe ear malformations where the endolymphatic duct failed to form correctly but still formed ear vesicles [23]. Very recently, Dode et al [24] established that loss-of-function mutations in *FGFR-1* underlie *KAL2* whereas a gain-of-function mutation in *FGFR-1* has been shown to cause a form of craniosynostosis.

### Bone morphogenic protein

Bone morphogenic proteins (BMP) have recently emerged as likely regulators of development of the permanent kidney (metanephros). Transcripts for BMPs and their receptors have been localized in the developing metanephros, but *BMP7* is the only Bmp that is expressed in the nephrogenic mesenchyme [20]. In vitro, *BMP2, 4*, and *7* have direct or indirect roles in regulation of ureteric branching morphogenesis and branch formation. In vivo, renal phenotypes have been reported in *BMP7* homozygous null mutant mice and *BMP4* heterozygous null mutant mice. In vitro, high concentrations of *BMP4* inhibited branching of the ureteric epithelium and changed its morphology, while nephrogenesis was inhibited by 50% [25]. *BMP4* (OMIM 112262) may be a physiologic regulator of the development of the periureteric smooth muscle layer and ureteric elongation. [26]. Furthermore, *BMP4* predominantly inhibits neural differenciation in early development. The vertebrate inner ear consists of a complex labyrinth of epithelial cells that is surrounded by a bony capsule. BMP proteins are important for the development of the otic epithelium in the chicken inner ear and possibly acting through BMP receptors IB (BMPRIB) are important for otic capsule formation. In addition, BMPs and their receptors influence chondrogenesis of the otic capsule, including the cartilage surrounding the semicircular canals [27].

### Wnt signaling and frizzled receptors

Wnts are a large family of secreted signal that regulate key morphogenetic steps during embryogenesis. Frizzled proteins have been identified as likely receptors for Wnt ligands in vertebrates and invertebrates. *Wnt-4* (OMIM 603490) is critical for genitourinary development but found only in the most distal collecting duct epithelium in the normal murine adult kidney. *Wnt-4* expression coincided with mesenchyme-to-epithelium transformation. In the metanephric kidney, *Wnt-4* is critically required for tubulogenesis in the pronephric kidney [28]. *Wnt-4* expression is induced throughout the collecting ducts in four murine models of renal injury that produce tubulointerstitial fibrosis: folic acid-induced nephropathy, unilateral ureteral obstruction, renal needle puncture, and polycystic kidney disease [29]. The expression of various components of the Wnt signaling pathway has been observed in the developing inner ear [30–33]. *Wnt-4 and frizzled* gene families are in the postnatal rat cochlea [34].

### GATA3 (OMIM 131320)

Recently, a member of the GATA-binding family of transcription factors was shown to be involved in the human hypoparathyroidism, sensorineural deafness and renal anomalies (HDR) syndrome. The HDR phenotype is consistent with the expression pattern of *GATA3* during human and mouse embryogenesis in the developing kidney (ureteric bud, collecting duct system, and mesangial cells), otic vesicle, and parathyroids. Terminal deletions of chromosome 10p result in that those studies in patients with 10p deletions have defined two nonoverlapping regions that contribute to the complex phenotype (includes hypoparathyroidism, heart defects, immune deficiency, deafness, and renal malformations or DiGeorge-like syndrome). These are the DiGeorge critical region II, which is located on 10p13-14, and the region for the HDR syndrome (Mendelian Inheritance in Man number 146255), which is located more telomeric (10p14-10pter) [35]. The apparently selective *GATA3* is further expressed in the developing spiral sensory neurons [36–38].

### $\alpha_8/\beta_1$ integrin

*ITGA8 (604063).* Using immunohistochemistry, Muller et al [39] demonstrated that in mouse, $\alpha_8/\beta_1$ integrin is expressed in many developing organs and particularly in the kidney, in mesenchymal cells bordering on epithelial cell sheets that undergo branching morphogenesis. By gene targeting, they produced mice lacking the $\alpha_8$ gene. The mutant mice showed severe deficits in kidney morphogenesis due to reduced growth of the ureteric bud toward the metanephric mesenchyme, reduced branching of the ureteric epithelium within the mesenchyme, and defective epithelialization of kidney mesenchymal cells. The authors [39] concluded that $\alpha_8/\beta_1$ integrin plays a crucial role in epithelial-mesenchymal interactions during kidney morphogenesis. Mechanosensory hair cells within the neuroepithelia of the cochlea and vestibule have stereocilia that are indispensable for mechanosensation. Evans and Muller [40] demonstrated that $\alpha_8/\beta_1$ integrin, its ligand fibronectin (135,600), and the integrin-regulated focal adhesion kinase (600,758) colocalize to the apical hair cell surface where stereocilia are forming. In mice homozygous for a targeted mutation of $\alpha_8/\beta_1$ integrin, the colocalization is perturbed, and hair cells in the utricle, a vestibular subcompartment, lack stereocilia or contain malformed stereocilia. Most $\alpha_8/\beta_1$ integrin-deficient mice die soon after birth due to kidney defects.

Many of the surviving mice have difficulty balancing, consistent with the structural defects of the inner ear. The authors [40] concluded that $\alpha_8/\beta_1$ integrin and potentially other integrins regulate hair cell differentiation and stereocilia maturation.

### The transcription factor FKH10 of the forkhead family

Forkhead(*Fkh) box 10* is expressed in the kidney mesenchyme and might function to locate the site where the ureteric bud forms on the Wolfian duct [20]. Although *Fkh10* may play a role in the kidney during later stages of development, it may be a minor, or perhaps redundant, role, as no kidney dysfunction was observed in homozygous knockout mice. On the contrary, *Fkh10* appears to be unique in the sense that it is an early otic vesicle-specific gene necessary for the development of both cochlea and vestibulum. These findings implicated *Fkh10* as an early regulator necessary for development of both cochlea and vestibulum and identified its human homolog *FKHL10* FKHL10 as a previously unknown candidate deafness gene at 5q34. The phenotype described by Hulander et al [41] resembles a group of human congenital inner ear malformations called "common cavity." The authors proposed that mutations in *FKH10* may cause a "common cavity" phenotype in humans.

### Subtype mitochondria-rich cells

Subtype A mitochondria-rich cells, from which protruding A mitochondria-rich cells are the activated state, are involved in proton secretion [apical H+-adenosine triphosphatase ATPase)] and thus are potential candidates for hearing loss accompanying renal tubular acidosis. Subtype B mitochondria-rich cells are the most likely candidates to be affected in Pendred syndrome because of the assumed function of pendrin as apical $Cl^-/HCO_3^-$ exchanger [42].

### Ear and kidney fluid homeostasis

Parchorin, a new chloride intracellular channel family member, preferentially resides in the epithelium of the ducts of the lacrymal, parotid, submandibular, and mammary glands and the pancreas, prostate, testis, and eye. In the kidney, parchorin was distributed mainly from the thick ascending limb to the distal convoluted tubule. Parchorin was also present in the cochlea and semicircular canal. The cellular distribution and changes in expression indicate that parchorin plays an important role, possibly in chloride transport, in the cells that create an ion gradient for water movement [43].

Prestin is a gene recently cloned from mammalian cochlear outer hair cells (OHC) using a single cell type, outer minus inner hair cell, specific suppressive substractive hybridization procedure. The localization and gene expression profile of the prestin protein fits the pattern of OHC's development of electromotility. When prestin is abundantly expressed in normally nonmotile kidney cells, nonlinear capacitance and motility that are normally only seen in OHCs can be recorded. Furthermore, both nonlinear capacitance and motility can be reduced by salicylate, a well-known inhibitor of electromotility. Prestin is the fifth member of a newly discovered anion transport family (SLC26), which are chloride-iodide transporters, $Cl^-/HCO_3^-$ exchangers or sulfate transporters [44].

The atrial natriuretic peptide (ANP) receptors are found within the human endolymphatic sac (ELS), with a predominance of ANP type B based on the intensity of staining. The ANPs may be involved in fluid homeostasis in the inner ear. Based on these findings, C-type natriuretic peptide may be a more effective peptide within the human ELS for fluid regulation because its binding affinity is virtually exclusive for the ANP type B receptor [45].

## CLINICAL APPROACH OF THE OTO-RENAL SYNDROMES

### Renal dysplasia or urinary tract anomalies and hearing loss

*BOR syndrome.* Although the association of branchial arch anomalies and hearing impairment has been known since the nineteenth century, the syndrome was first outlined in 1975 [46]. The incidence of BOR syndrome is 1:40,000 and has been reported to occur in 2% of profoundly deaf children [47]. Typical signs are hearing impairment, ear malformations, branchial cleft, and renal abnormalities. The clinical expression of the BOR syndrome gene is extremely variable from one family to the next [48–51]. Clinical studies suggest that there are at least three distinct syndromes involved in the branchiogenic disorders. The first, BOR dysplasia, is associated with renal anomalies; but the second, BO dysplasia, lacks renal anomalies; and the third, BR anomalies, results in branchial and renal disorders with no associated hearing loss. Otic anomalies affect all three compartments of the ear (preauricular pits and appendages (77% of cases), auricular malformations, and atresia to stenosis of the external auditory canal), cervical fistulas and cysts (63% of cases) representing developmental defects of the first branchial arches (for the tympanic cavity), as well as the first and second branchial arches (ossicular) and the inner ear (absent or underdevelop cochlea and absent or hypoplastic semicircular canals). Thus, the hearing loss of the syndrome could be conductive (30%) (caused by outer and middle ear anomalies), sensorineural (20%) (caused by inner ear anomalies), or mixed (50%). The severity of the impairment may differ between the two ears [10]. Facial nerve paralysis has also been reported in association [52]. In BOR syndrome middle and inner

ear defects are commonly ossicular chain malformations [52] and Mondini dysplasia of the cochlea [52, 53]. The renal defects, estimated to be severe in only approximately 6% of heterozygotes, include collecting system duplications, renal hypoplasia, cystic dysplasia, and agenesis [47, 54], characterized by decreased renal volume and size, loss of ultrasound normal corticomedullary differentiation, and hyperechogenecity of the renal cortex. Histologically, there is glomerular hyalinization, mesangial proliferation, and basement membrane splitting [55]. Bilateral renal agenesis is the extreme, leading to miscarriage or immediate neonatal death [56]. Other renal abnormalities include hydronephrosis associated with ureteropelvic junction obstruction or vesicoureteral reflux. There can also be bifid kidneys with double ureters and calyceal anomalies [6]. Other minor renal anomalies were subsequently reported [57, 58] and radiographically documented in 13% [47]. Similar to BOR syndrome, *Eya1*$^{+/-}$ mice also showed renal defects at low penetrance, including renal hypoplasia (2/25 in 129 newborns) and unilateral agenesis (2/11 in BL/6 adult mice). In contrast to the low incidence of renal defects in *Eya1*$^{+/-}$ mice, all *Eya1*$^{-/-}$ mice lacked kidneys and ureters due to a failure of ureteric bud outgrowth and metanephric induction. [59]. The gene underlying this syndrome, *EYA1* (OMIM 601653), is homologous to the *Drosophila* developmental gene eyes absent wich encodes a transcriptional coactivator required for eye specification. The first BOR syndrome gene has been mapped to chromosome 8q [60, 61]; and the *EYA1* gene (homologous to the *Drosophila* developmental gene denoted as "eyes absent") underlying this syndrome has been identified, and several mutations have been reported [62, 63]. The *EYA1* gene (OMIM 601653) expressed very early, between 4 and 6 weeks of human development, can be correlated with the branchial fistulas, sinuses and cysts but not with the outer and middle ear anomalies. Deafness relates to abnormalities in the three ossicles of the middle ear derived from the first and second branchial arches, while the branchial fistulas relate to abnormalities of the second, third, and fourth arches. In contrast, *Eya1* is expressed during the slightly more advanced stage of outer and middle ear morphogenesis at E13.5, in the mesenchyme adjacent to the first branchial cleft and surrounding the primordia of the middle ear ossicles, and in the epithelium of the tubotympanic recess. During early inner ear development, *Eya1* is expressed in the ventromedial wall of the otic vesicle in the statoacoustic ganglion, and in the periotic mesenchyme, consistent with the cochlear anomalies and sensorineural hearing loss of BOR syndrome. Subsequently, *Eya1* expression is observed in the differentiating hair and supporting cells of the sensory epithelia, as well as in the associated ganglia, and persists after differentiation has taken place. *Eya1* expression in the condensing mesenchymal cells of the kidney is consistent with the excretory and collecting system anomalies of BOR syndrome [8]. In the embryonic human kidney, the *EYA1* gene is expressed strongly, and in the BOR syndrome there is an inductive fault between the ureteric bud and the metanephric mesenchymal mass as the ureteric bud branches into the renal parenchyma. Haploinsufficiency for human *EYA1*, a homologue of the *Drosophila* melanogaster gene eyes absent (eya), results in the dominantly inherited disorders, which are characterized by craniofacial abnormalities and hearing loss with (BOR) or without [branchio-oto (BO)] kidney defects syndrome [62, 64, 65]. *Eya1* heterozygotes show renal abnormalities and a conductive hearing loss similar to BOR syndrome, whereas *Eya1* homozygotes lack ears and kidneys due to defective inductive tissue interactions and apoptotic regression of the organ primordia [66]. Inner ear development in *Eya1* homozygotes arrests at the otic vesicle stage. Deficiency for the Wilm's tumor gene WT-1 results in kidney abnormalities similar to those seen in *Eya1*$^{-/-}$ mice [67], and *WT-1* expression, normally present in the metanephric mesenchyme and urogenital ridge, was unaffected in *Eya1* mutants. In contrast, expression of the gene encoding glial-derived neurotrophic factor (*Gdnf*) [68, 69], identified as a mesenchyme-derived signal that acts as a ligand for the c-ret receptor tyrosine kinase expressed at the tip of the ureteric bud [70–72], was not detected in *Eya1* homozygotes. Thus *Eya1* also appears to operate in the genetic regulatory cascade controlling ureteric bud outgrowth, upstream of *Gdnf*. *Eya4* is also thought to have a role in ear formation, as its expression has been reported in neuroepithelia of rat inner ear from E14.5 onwards, and loss of *Eya4* in humans causes defaness [73, 74]. However, some families are not linked to the *EYA1* locus, suggesting that BOR syndromes may also be due to mutations in genes other than the *EYA1* gene.

*TBS*. Townes and Brocks first described the syndrome in 1972 [75] (OMIM 107480), as a rare autosomal-dominant malformation syndrome with multiple malformations and variable expression. Diagnostic criteria suggested for TBS include two or more of the following: anorectal malformation (47%) (imperforate anus, anteriorly placed anus, anal stenosis); hand malformations (45%) (preaxial polydactyly, triphalangeal thumb, bifid thumb); andexternal ear malformation (71%) with deafness [76] and renal malformations. Renal ear anal radial (REAR) syndrome has also been a term used to describe this condition [77]. Intelligence is usually normal, although mild-to-moderate mental retardation has been reported [78, 79]. Other clinical features include heart (lethal truncus arteriosus, pulmonary valve atresia) [80] and eye abnormalities (coloboma) [75]. External ear anomalies in TBS typically include small ears with an overfolded superior helix and small anthelix, sometimes cupped, with preauricular tags or

pits. Other descriptive terms reported for ear shape include microtia, "satyr" and "lop." Sensorineural hearing loss is common in TBS, ranging from mild to profound. It is usually congenital and primarily sensorineural, although a small conductive component is often present. At least in some patients it is progressive, and is worse in the high frequencies [81]. Structural middle and inner ear anomalies have been reported and include hypoplastic malleus head, uncudomalleolar fusion, abnormally shaped oval window and incus and anomalies of the semicircular canals [82, 83]. A high incidence of genitourinary abnormalities is founds in TBS. These include unilateral or bilateral hypoplastic or dysplastic kidneys, horeshoe kidney, renal agenesis, multicystic kidney, posterior urethral valves, vesicoureteral reflux, and meatal stenosis [75, 84, 85]. Renal failure or impaired renal function were common. Several patients have had renal transplantation [81, 85]. This underscores the need for renal imaging and monitoring of renal function in TBS patients. A prominent midline perineal raphe or bifid scrotum or both is common in males with TBS. Hypospadias has also been reported [abstract; Friedman PA et al, *Am J Hum Genet* 41 (Suppl):A60, 1987] [81, 84].

TBS is caused by a defect in the gene encoding the *SALL1* putative transcription factor, a protein possibly required for urologic, renal, limb, ear, brain, and liver development. *SALL1* was mapped to 16q12.1 [abstract; Friedman PA et al, *Am J Hum Genet* 41 (Suppl):A60, 1987]. It is caused by mutations in a putative $C_2H_2$ zinc-finger transcription factor [86]. In the forelimb, *SALL1* haplo insufficiency in the most severe cases leads to additional triphalangeal thumbs, which resemble fingers more than thumbs. *SALL1* could therefore function as a direct or indirect repressor of joint formation and finger outgrowth. Because most duplications do not involve metacarpal bones, *SALL1* seems to be especially important for the later steps in distal limb differentiation and thumb specification during the seventh to eighth weeks of embryonic development. *SALL1* may be required during later steps in development of the outer, middle and inner ear—more specifically, for differenciation of the first and second branchial arches and for differenciation of the otic vesicle. *SALL1* therefore seems to be required for some of these steps during the fifth to eighth week of embryonic development [86]. Renal agenesis or multicystic kidneys represent the most severe kidney anomalies in TBS. Renal agenesis is thought to result from insufficient outgrowth of the ureteric buds, whereas multiple cysts can develop due to either failure of nephrons to contact the collecting tubules or failure of superfluous tubules to degenerate or malformation of collecting tubules.

*Okihiro syndrome.* Okihiro syndrome refers to the association of forearm malformations with Duane syndrome of eye retraction. Specifically, there is overlap of clinical features with other conditions, most notably Holt-Oram syndrome, a condition resulting from mutation of the *TBX5* locus and TBS, known to be caused by mutations in the *SALL1* gene. Kohlhase et al [87] have characterized the human *SALL4* gene on chromosome 20q13.13-q13.2.

*Kallmann-De Morsier syndrome.* Kallmann syndrome is a developmental disease characterized by gonadotropin-releasing hormone (GnRH) deficiency and olfactory bulb hypoplasia. The gene underlying the X chromosome-linked form, *KAL-1*, has been identified for several years that encoded protein, *anosmin-1*, is a transient and regionally restricted component of extracellular matrices during organogenesis in man. *Anosmin-1* (OMIM 308700) was detected in the basement membranes and/or interstitial matrices of various structures, including mesonephric tubules and duct, branches of the ureteric bud, and inner ear [88].

Kallmann syndrome refers to the association of hypogonadotrophic hyogonadism with anosmia (or hyposmia). The anosmia has been related to the absence or hypoplasia of the olfactory bulbs and olfactory tracts [89]. Three different modes of inheritance have been reported in familial cases in Kallmann syndrome, X chromosome-linked (*KAL-1*, OMIM 308700, the most frequent gene responsible localized to Xp 22.3 region), autosomal-dominant (*KAL-2*, OMIM 147950), and autosomal-recessive (*KAL-3*, OMIM 244200). The encoded protein was named anosmin-1 in reference to the defective sense of smell characterizing the disease. The presence of *anosmin-1* in the inner ear structures at early developmental stages suggests that the defect underlying the hearing loss in X-linked Kallmann syndrome occurs during organogenesis. Moreover, the regional distribution of the protein argues against a direct role in the differentiation of the cochlear sensory epithelium. The observation that *anosmin-1* is produced in the urinary system from the early steps of nephrogenesis strongly suggests that the renal aplasia in X-linked Kallmann syndrome results from actual agenesis of the metanephros and not from degeneration after the organogenesis period. Moreover, the restriction of the protein to the collecting duct system argues in support of the absent development or early degeneration of this system (which is normally required for induction of the metanephrogenic mesenchyme) as the primary defect. This is further supported by the absence of the vas deferens ipsilateral to the missing kidney, which has been observed in a few affected individuals [90, 91]. This anomaly results from the degeneration of the mesonephric duct (from which the ureteric bud is derived), a structure that also produces *anosmin-1*. A direct role of *anosmin-1* in the reciprocal mesenchymal-epithelial interactions between the renal blastema and ureteric branches appears unlikely since, at any studied stage, anosmin-1 was only detected in

proximal segments of the ingrowing ureteric arborization and not in the distal branches which are induction sites. Finally, the incomplete penetrance of the renal aplasia in X-linked Kallmann syndrome, which does not exceed one out of two or three cases [90], as well as the presence of one functional kidney in affected individuals, both indicate that other molecules can compensate for *anosmin-1* deficiency in kidney organogenesis. Hearing loss has been reported in many individuals affected by Kallmann syndrome [92] and, more recently, in several authenticated X-linked cases of the disease [89]. In most affected individuals, the hearing loss is described as bilateral and perceptive but also as conductive hearing loss [93]. However, detailed functional and morphologic investigations have been reported in only a small number of patients and, thus far, in not individual with authentificated X-linked Kallmann syndrome. Very recently, Dode et al [24] reported that a second gene has been recently identified that encodes for *FGFR-1*. The authors established that loss-of-function mutations in *FGFR-1* underlie *KAL-2* whereas a gain-of-function mutation in *FGFR-1* has been shown to cause a form of craniosynostosis. Moreover, the authors suggest that the *KAL-1* gene product, the extracellular matrix protein *anosmin-1*, is involved in FGF signaling and propose that the gender difference in anosmin-1 dosage explains the higher prevalence of the disease in males.

### Renal tubular disturbance and sensorineural deafness

*Bartter syndrome*. Renal salt loss in Bartter syndrome is caused by impaired transepithelial transport in the loop of Henle. Sodium chloride is taken up apically by the combined activity of NKCC2 ($Na^+$-K-–2 $Cl^-$ cotransporters) and ROMK potassium channels. Chloride ions exit from the cell through basolateral ClC-Kb chloride channels. Mutations in the three corresponding genes have been identified [50–52] that correspond to Bartter syndrome types 1 to 3. A gene locus of a fourth variant of antenatal BS called BSND (Bartter with sensorineural deafness), which in contrast to the other forms is associated with sensorineural deafness (SND) and renal failure, has been mapped to chromosome 1p [94] and has resulted in new insights into renal salt handling, diuretic action and blood-pressure regulation [95, 96]. Positional cloning of the Bartter sensorineural deafness (*BSND*) gene, which underlies Bartter syndrome type 4 (also named BSND; OMIM 602522), identified *barttin* (OMIM 606412). In situ hybridization showed barttin expression in specific nephron segments and in the stria vascularis [95]. Barttin forms heteromers with ClC-K1 (ClC-Ka) in the thin ascending limb of Henle [97] and with ClC-K2 (ClC-Kb) in the thick ascending limb and more distal segments [98]. *Barttin* was also detected in basolateral membranes of intercalated cells of the collecting duct, which are known to express ClC-K2 (ClC-Kb) as well [98]. In the inner ear, *barttin* colocalized with ClC-K in $K^+$ secreting marginal cells of the stria vascularis. The basolateral staining for both proteins contrasts with the apical localization of the KCNQ1 $K^+$ channel. *Barttin* was also found in $K^+$ secreting vestibular dark cells, where it colocalizes in basolateral membranes with ClC-K below apical membranes that express KCNQ1. In the kidney, ClC-K/barttin heterodimers mediate $Cl^-$ reabsorption by facilitating its basolateral efflux. In the stria, ClC-K/barttin channels drive K+ secretion by recycling $Cl^-$ for the basolateral NKCC1 cotransporter. This role is analogous to that of ROMK in $Cl^-$ reabsorbing cells of the thick ascending limb, where it recycles $K^+$ for the apical NKCC2 cotransporter. *Barttin* acts as an essential β-subunit for ClC-Ka and ClC-Kb chloride channels, with which it colocalizes in basolateral membranes of renal tubules and of potassium-secreting epithelia of the inner ear. Disease-causing mutations in either ClC-Kb or barttin compromise currents through heteromeric channels. ClC-Ka and ClC-Bb are higly homologous chloride channels that are nearly exclusively expressed in kidney. CLCNKB mutation in Bartter syndrome [99] together with immunohistochemical results [98, 100] suggest that human ClC-Kb (the ortholog of rodent ClC-K2) mediates basolateral chloride efflux in the thick ascending limb of Lembe's loop and in more distal nephron segments. Similarly, immunolocalization [95] and the diabetes insipidus observed in $Clcnk1^{-/-}$ mice [101] indicate that rodent ClC-K1 (the ortholog of human CC-Ka) is crucial for transepithelial transport in the thin ascending limb. Because *barttin* is crucial for ClC-Kb function, its inactivation results in renal salt wasting as do mutations in ClC-Kb [102]. However, because *barttin* also associates with ClC-Ka, additional symptoms are expected. These may resemble the diabetes insipidus-like phenotype observed in $Clcnk1^{-/-}$ mouse [101]. Indeed, BSND patients present with more severe renal symptoms than patients having mutations in ClC-Kb [95, 103]. Unlike mutations in *barttin* [104], mutations in the ClC-Kb β-subunit [99] do not cause deafness, nor was deafness described in mice disrupted for ClC-K1 [101].

*Renal tubular acidosis with progressive nerve deafness (267300)*. Primary distal renal tubular acidosis (dRTA) type I is a hereditary renal tubular disorder, which is characterized by impaired renal acid secretion resulting in metabolic acidosis. Clinical symptoms are nephrocalcinosis, nephrolithiasis, osteomalacia, and growth retardation. Biochemical alterations consist of hyperchloremic metabolic acidosis, hypokalemia with muscle weakness, hypercalciuria, and inappropriately raised urinary pH. Autosomal-dominant and rare forms of recessive dRTA (rdRTA) are known to be caused by mutations in the gene for the anion exchanger AE1. Two types of rdRTA have been differentiated by the presence or absence of

sensorineural hearing loss, but appear otherwise clinically similar. A large proportion of autosomal-recessive distal renal tubular acidosis (RTA) is associated with mutations in the *ATP6B1* gene (192132) encoding the B1 subunit of H(+)-ATPase [105]. H(+)-ATPase is one of the key membrane transporters for net acid excretion in the α-intercalated cells of the medullary collecting duct and was mapped to 2cen-q13. Sensorineural hearing loss frequently accompanies this type of distal RTA. Karet et al [106] demonstrated that distal renal tubular acidosis with sensorineural hearing loss (dRTA) is caused by mutations in the *ATP6B1* gene, which encodes the B subunit of the apical proton pump mediating distal nephron acid secretion. Consistent with the associated hearing loss, Karet et al [106] demonstrated expression of *ATP6B1* in the cochlea and endolymphatic sac. This demonstration, together with the known requirement for active proton secretion to maintain proper endolymph pH, implicated *ATP6B1* in endolymph pH homeostasis and in normal auditory function. The authors studied the dRTA syndrome in four outbred kindreds with two or more affected sibs and in 27 kindreds with parental consanguinity, of which seven had more than one affected individual. Of the 27 consanguineous kindreds, parents were first cousins in 20 and more distantly related in the remainder. All index cases were diagnosed by 6 years of age, with 19 diagnosed by 1 year of age. They presented either acutely with dehydration and vomiting, or with failure to thrive and/or growth impairment. In each case, the diagnosis was based on inappropriately alkaline urine (pH greater than 5.5) and the presence of systemic metabolic acidosis with normal anion gap, evidence of renal potassium wasting, and no evidence of secondary causes of dRTA. All patients had nephrocalcinosis, accompanied by elevated urinary calcium. Rickets was also noted in five of these kindreds. Despite the nephrocalcinosis, renal function was otherwise normal in every case, and remained so in all but one female who developed end-stage renal disease at 18 years of age; the median follow-up was 5 years (range 0.5 to 40). All patients had normal serum sodium, calcium, phosphate, creatinine, and magnesium. Bilateral sensorineural hearing loss was found in 15 affected subjects from 10 kindreds. The hearing loss varied in severity from mild (40 dB) to profound (100 dB). In 20 subjects from 15 kindreds, audiometry excluded a sensorineural deficit. Significantly, hearing status in all 10 tested sib pairs or trios with dRTA was concordant, suggesting that the occurrence of hearing impairment was not stochastic among affected patients.

Mutations in the gene encoding the apical pump *ATP6B1* cause dRTA accompanied by deafness. However, ATP6N1B mutations, which were expressed exclusively to the apical surface of -intercalated cells in the kidney but not other main organs, do not cause deafness [107].

*Hypophosphatemia, hereditary, type II, or hypophosphatemic D–resistant rickets II; HPDR II; HYP2; Gyro equivalent; Gy equivalent (307810).* In the mouse, Lyon et al [108] identified a second type of X-linked dominant hypophosphatemia. The mutation, called Gyro (*Gy*), is manifested by rickets/osteomalacia as in the *Hyp* mutation (307800) but in addition shows circling behavior, inner ear abnormalities, sterility in hemizygous males, and a milder phenotype in heterozygous females. The *Gy* and *Hyp* mutations have similar expression in the renal tubule but the *Gy* mutation has an additional effect on the inner ear. The nature of the translation product that is common to the inner ear and renal brush-border membrane is unknown. The *Gy* allele is expressed in the inner ear of some heterozygous mice, which show circling behavior. Deafness has been reported in humans with X-linked hypophosphatemia [109, 110] but has been observed also in the autosomal-recessive form of hypophosphatemic rickets [111]. It is likely that an X-linked human homologue of the mouse *Gy* mutation will be identified. That there are at least two forms of X-linked hypophosphatemia determined by genes on Xp11.22 is indicated by the fact that causative mutations have been found both in the *HYP* gene, also known as *PEX* (307800), which has homologies to the endopeptidases and in the *CLCN5* gene (300008) [112]. Strom et al [113] cloned the mouse homologue of the *PEX* gene, which was known to be the site of mutations causing hereditary hypophosphatemia in the human. They found a high degree of conservation between man and mouse.

## Glomerular disease and deafness

*Alport syndrome.* Alport syndrome, a hereditary basement membrane disease affecting approximately one in 5000 people, is a disorder of type IV collagen with progressive nephropathy, ocular abnormalities, and high-tone sensorineural deafness. In X-linked Alport syndrome, mutations in the *COL4A5* gene encoding the α5 chain of type IV collagen lead to loss of the α3/α4/α5 network and increased susceptibility of the glomerular basement membrane to long-term damage. No effective drug therapy exists for the disease, which is currently treated by dialysis and renal transplant [114, 115]. The classic phenotype as described by Alport [116] affected both genderss in successive generations. The renal disease becomes evident as recurrent microscopic or gross hematuria as early as childhood, earlier in males than in females. Nephrotic syndrome, although unusual, has been reported. The most common form of the disease is X-linked, and caused primarily by mutations in the *collagen α5(IV)* gene [117], accounting for ~ 80% of the cases. Mutations in the *collagen α3(IV)* or *α4(IV)* genes lead to the recessive forms of the diseases [118, 119]. The absence of any one of these type IV collagen chains can result in

the absence of all three chains in the glomerular basement membrane, presumably due to an obligatory association of the three chains in forming the type IV collagen superstructure [119, 120]. Normal distribution of the three α chains is observed in approximately one third of patients [121]. Expansion of the mesangial matrix occurs early in Alport renal pathogenesis. Hearing loss, which is sensorineural and primarily affects high tones, occurs in 30% to 50% of relatives with renal disease. The severity of auditory and renal features does not correlate in a given individual. The molecular defects that underlie the otopathology in this disease remain poorly understood. Using a canine model of X-linked Alport syndrome to determine the expression of type IV collagen α chains in the inner ear, Harvey et al [122] show that affected dogs have complete absence of the α3/α4/α5 network. The lateral aspect of the spiral ligament is populated by tension fibroblasts that express α-smooth muscle actin and nonmuscle myosin and are postulated to generate radial tension on the basilar membrane via the extracellular matrix for reception of high frequency sound. The authors propose that in Alport syndrome, the loss of the α3/α4/α5 network eventually weakens the interaction of these cells with their extracellular matrix, resulting in reduced tension on the basilar membrane and the inability to respond to high frequency sounds. Demonstration of linkage analysis to chromosome 2q in consanguineous families and of mutations in one or the other of these two autosomal genes provided clear evidence of the existence of the autosomal recessive form of Alport syndrome. It remained to be determined whether mutations in either of these genes in heterozygous state cause abnormality. Jefferson et al [123] mapped autosomal-dominant Alport syndrome in a single family to chromosome 2, in the vicinity of *COL4A3* and *COL4A4*. Furthermore, in the autosomal-dominant Alport syndrome, a splice-site mutation resulting in skipping of exon 21 of the *COL4A3* gene was detected by van der Loop et al [124].

*Fechtner syndrome*. The first study reports by Peterson in a family comprising four generations in whom nephritis, deafness, congenital cataracts, macrothrombocytopenia, and leukocyte inclusions were observed in varying combinations in eight of 17 members [125]. Fechtner syndrome (FTNS) is a rare inherited condition characterized by progressive nephritis (39%), macrothrombocytopenia (100%), Dohle-like leukocyte inclusions (100%), deafness (49%), and cataract (54%). Deafness was high-tone sensorineural. Renal disease ranged from microscopic hematuria to end-stage renal failure necessitating dialysis and kidney transplantation. Although it recently was shown that FTNS derives from mutation of *MYH9*, the gene for the heavy chain of nonmuscle myosin IIA (NMMHC-IIA) [126]. Hearing loss in Fechtner syndrome appears to be sensorineural with the higher frequencies primarily affected. The most striking difference between hearing loss in Fechtner syndrome and that in Alport syndrome was that the vast majority of hearing disorders in the latter occur in male patients, which is not the case in Fechtner syndrome. Hearing loss in Fechtner syndrome develops from the second decade of life and progresses slowly with several episodes of sudden deafness [127]. Renal biopsy findings are consistent with those of Alport syndrome, and the associated renal disease is said to be unusual before middle to late adulthood. Chronic renal failure can occur at a young age in patients with Fechtner syndrome, with a possible relation to race/ethnicity. Fechtner syndrome, or variants of Alport syndrome, should be considered in patients presenting with proteinuria and thrombocytopenia [128].

Because of the phenotypic similarities between May-Hegglin anomaly and Fechtner syndrome and the demonstration that the two disease loci are mapped to overlapping regions on chromosome 22, the May-Hegglin/Fechtner Syndrome Consortium (2000) suggested that they, as well as Sebastian syndrome, might be allelic. *MYH9* was a strong candidate gene because it is located within that region of chromosome 22, is expressed in platelets, and is upregulated during granulocyte differentiation.

## PERSPECTIVES

We have described ear and kidney clinical syndromes and their molecule expression. Furthermore, other gene or transcription factors are responsible for physiologic symptoms in one or both organs but without yet discribed clinical syndromes. These pathologies are described in the following paragraphs.

First, Pendred syndrome (PDS) (274600), the most common syndromal form of congenital deafness, is an autosomal-recessive disorder associated with developmental abnormalities of the cochlea, fluctuating and progressive sensorineural hearing loss, and goiter. This syndrome was described by Vaughan Pendred [129]. It was exactly a century later that Coyle et al [130] and Sheffield et al [131] showed that the disorder maps to chromosome 7q (22-q31). The Solute Carrier Family 26, Member 4; (*SLC26A4*) (605646) gene encodes an anion transporter known as pendrin (OMIM 605646) and is the gene mutant in PDS (274600), DFNB4 (600791), and enlarged vestibular aqueduct syndrome (EVA) (603545) [132]. *Pendrin* is an anion transporter encoded by the Pendrin Syndrome (PDS/Pds) gene. Labeling was detected on the apical surface of a subpopulation of cells within the cortical collecting ducts that also express the $H^+$-ATPase but not aquaporin-2 (AQP-2), indicating that pendrin is present in intercalated cells of the cortical collecting ducts. Furthermore, pendrin was detected exclusively within the subpopulation of intercalated cells that express the H+-ATPase but not the AE1 and that are

thought to mediate bicarbonate secretion. The same distribution of *pendrin* was observed in mouse, rat, and human kidney. RNA in situ hybridization studies revealed that Pds is expressed in discrete areas of the mouse inner ear, including regions thought to play a key role in fluid transport [133]. *Pendrin* directly mediates the transport of bicarbonate across the apical membrane of intercalated cells. *Pendrin* is capable of chloride/bicarbonate exchange [134]. An alternate explanation is that pendrin might play an indirect role in apical bicarbonate transport in intercalated cells [135]. Neither PDS patients nor pendrin-deficient mice have been reported to develop overt acid-base disturbances, such as a metabolic alkalosis. This likely reflects the fact that the kidney has other means of regulating bicarbonate excretion in the absence of *pendrin* (e.g., by regulation provided by the $Na^+/H^+$ exchanger NHE3 in the proximal tubule and the loop of Henle [136]). However, there is no yet clinical corresponding pathology in humans.

Second, *KCNQ* genes encode a growing family of six transmembrane domains, single pore-loop, K(+) channel α subunits that have a wide range of physiologic correlates. Mutations in the channel *KCNQ1* can cause one form of inherited long QT syndrome (LQT1), as well as being associated with a form of deafness. *KCNQ2* and *KCNQ3* heteromultimers mutations may cause an inherited form of juvenile epilepsy. The *KCNQ4* gene encoding to the potassium concentration in outer hair cells of the cochlea and in type I hair cells of the vestibular apparatus, mutations in which lead to a form of inherited deafness, and possibly, mutations in *KCNQ5* lead to a form of inherited retinal degeneration (KCNQ5) [137]. Hearing depends on a high K(+) concentration bathing the apical membranes of sensory hair cells. K(+) that has entered hair cells through apical mechanosensitive channels is transported to the stria vascularis for re-secretion into the scala media. K(+) probably exits outer hair cells by KCNQ4 K(+) channels, and is then transported by means of a gap junction system connecting supporting Deiters' cells and fibrocytes back to the stria vascularis. Mice lacking the K(+)/Cl(−) (K-Cl) cotransporter Kcc4 are deaf because their hair cells degenerate rapidly after the beginning of hearing. In the mature organ of Corti, Kcc4 is restricted to supporting cells of outer and inner hair cells. Similar to some human genetic syndromes, deafness in Kcc4-deficient mice is associated with renal tubular acidosis. It probably results from an impairment of Cl(−) recycling across the basolateral membrane of acid-secreting α intercalated cells of the distal nephron [138].

Third, in the kidney, at least seven aquaporins are expressed at distinct sites. AQP-2 is exclusively expressed in the principal cells of the connecting tubule and collecting duct and is the predominant vasopressin-regulated water channel. Body water balance is tightly regulated by vasopressin, and multiple studies now have underscored the essential roles of AQP-2 in this. Vasopressin regulates acutely the water permeability of the kidney collecting duct by trafficking of AQP-2 from intracellular vesicles to the apical plasma membrane. Lack of functional AQP-2 is seen in primary forms of diabetes insipidus, and reduced expression and targeting are seen in several diseases associated with urinary concentrating defects such as acquired nephrogenic diabetes insipidus, postobstructive polyuria, as well as acute and chronic renal failure. In contrast, in conditions with water retention such as severe congestive heart failure, pregnancy, and syndrome of inappropriate antidiuretic hormone secretion, both AQP-2 expression levels and apical plasma membrane targetting are increased, suggesting a role for AQP-2 in the development of water retention [139]. AQP-2 is expressed in the cochlea, testis, and kidney and an absence of tissue-specific splice variants. The level of AQP-2 transcript in the cochlea was tenfold lower relative to its expression in the testis and kidney. In the rat and mouse cochlea, AQP-2 was expressed in the structures bordering the endolymph, including Reissner's membrane, the organ of Corti, inner and outer sulcus cells and the spiral limbus. The physiologic role of AQP-2 in water transport and its expression pattern in the cochlea suggests an important role for AQP-2 in fluid homeostasis of the inner ear [140]. Nephrogenic diabetes insipidus, which can be inherited or acquired, is characterized by an inability to concentrate urine despite normal or elevated plasma concentrations of the antidiuretic hormone arginine vasopressin. Polyuria, with hyposthenuria, and polydipsia are the cardinal clinical manifestations of the disease. About 90% of patients with congenital nephrogenic diabetes insipidus are males with the X-linked recessive form of the disease (OMIM 304800) who have mutations in the arginine vasopressin receptor 2 gene (*AVPR2*), which encodes for the vasopressin V2 receptor. The gene is located in chromosomal region Xq28. In <10% of the families studied, congenital nephrogenic diabetes insipidus has an autosomal-recessive or autosomal-dominant (OMIM 222000 and 125800, respectively) mode of inheritance. Mutations have been identified in the aquaporin-2 gene (*AQP2*), which is located in chromosome region 12q13 and encodes for the vasopressin-sensitive water channel [141, 142].

Finally, kidney and inner ear have specialized epithelia involved in active ion transport, which are separated from the vessels by a basement membrane of similar composition. Meyer, Balz, and Felix [143] indicate that the glomerular and the stria vascularis basement membrane are simultaneously affected in early stages in the *Mpv17*-negative mouse strain. Concomitant deposits of IgG during the progressive development of the disease support the idea of a shared antigen.

The *Mpv17* mouse strain is a recessive transgenic mouse mutant that develops glomerulosclerosis and

nephrotic syndrome at a young age. These revealed degeneration of the stria vascularis and spiral ligament, loss of cochlear neurons and degeneration of the organ of Corti [144]. The alterations observed here were similar to those described for Alport syndrome. These findings indicate that, although the molecular cause is different, the *Mpv17* mouse model may share pathologic mechanisms involved in patients with Alport syndrome [144].

## CONCLUSION

Ear and kidney development have been characterized in great detail, and we now know that embryologically, ear and kidney primordia arise at different times and develop at different rates. Gene expression studies allow a better understanding of ear and kidney clinical syndrome. A careful analysis of those clinical manifestations will also help us in the comprehension of ear and kidney development.

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